UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MAY 22 1989

MEMORANDUM

SUBJECT: Isofenphos (Oftar) - Review of Two Mutagenicity Studies (EPA Reg ration No. 3125-326)

TOX Chem No.: 447AB
HED Project No.: 9-1007
MRID Nos.: 410088-01, 410088-02

FROM: Yiannakis M. Ioannou, Ph.D., Acting Section Head
Review Section I, Toxicology Branch II (HFAS)
Health Effects Division (H7509C)

TO: William H. Miller, PM 16
Insecticide-Rodenticide Branch
Registration Division (H7505C)

THRU: Marcia van Gemert, Ph.D., Acting Chief
Toxicology Branch II (HFAS)
Health Effects Division (H7509C) M. van Gemert 5/18/89

Registrant: Mobay Corporation, Kansas City, MO

Action Requested

Review two mutagenicity studies (with oftanol) titled:

1. Chromosomal Aberrations in Chinese Hamster Ovary (CHO) Cells, and

2. Unscheduled DNA Synthesis in Rat Primary Hepatocytes.

Summary and Conclusions

1. Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells

CHO cells were exposed to Oftanol technical (91% ai) at dose levels of 0, 0.02, 0.04, 0.08, and 0.16 uL/mL in the presence or absence of metabolic activation. Although slight cytotoxicity was seen with the three highest dose levels, there was no increase in the frequency of aberrations per cell at any dose level tested with or without S9 activation. The study is Acceptable.

2. Unscheduled DNA Synthesis in Rat Primary Hepatocytes

Rat hepatocyte cultures were exposed for 18 to 20 hours to Oftanol at dose levels of 0.001, 0.003, 0.01, 0.02, and 0.03 uL/mL. Although cytotoxicity was observed for the two highest dose levels, none of the dose levels tested resulted in a significant increase in net nuclear grain counts. Therefore, Oftanol was not genotoxic. The study is Acceptable.

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Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	Toxicity Category	CORF Grade/ Doc. No.
Mutagenicity - Cytogenetic Assay in CHO Cells; Microbiological Associates, Inc.; #T8298-337; February 6, 1989	Oftanol Technical (91% ai)	410088-01	Not clastogenic with or without metabolic activation at dose levels ranging from 0.02 to 0.16 uL/mL.		Acceptable
Mutagenicity - UDS in Rat Hepatocytes; Microbiological Associates, Inc.; #T8298-380; February 10, 1989	Oftanol Technical (91% ai)	410088-02	Negative for UDS in primary rat hepatocytes at dose levels ranging from 0.001 to 0.03 uL/mL.		Acceptable

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EPA No.: 68D80056
DYNAMAC No.: 175-B
TASK No.: 1-75B
May 8, 1989

DATA EVALUATION RECORD

OFTANOL

Mutagenicity--Unscheduled DNA Synthesis in Primary Rat Hepatocytes

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: 5/8/89

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EPA No.: 68D80056
DYNAMAC No.: 175-B
TASK No.: 1-75B
May 8, 1989

DATA EVALUATION RECORD

OFTANOL

Mutagenicity--Unscheduled DNA Synthesis in Primary Rat Hepatocytes

REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E. McCarroll
Date: 5-8-89

I. Cecil Felkner, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: Robert Weir Jr. I. Cecil Felkner
Date: 5-8-89

APPROVED BY:

Roman J. Pienta, Ph.D.
Department Manager
Dynamac Corporation

Signature: Roman J. Pienta
Date: 5-8-89

Mike Ioannou, Ph.D.
EPA Reviewer and
Acting EPA Section Head
Toxicology Branch II (H-7509C)

Signature: Mike Ioannou
Date: 5-10-89

DATA EVALUATION RECORD

CHEMICAL: Oftanol.

STUDY TYPE: Unscheduled DNA synthesis in primary rat hepatocytes.

MRID NUMBER: 41088-02.

TEST MATERIAL: Oftanol technical.

SYNONYM(S)/CAS NO.: 25311-71-1.

SPONSOR: Mobay Corp., Stilwell, KS.

TESTING FACILITY: Microbiological Associates, Inc., Rockville, MD.

TITLE OF REPORT: Unscheduled DNA Synthesis in Rat Primary Hepatocytes: Test Article: Oftanol Technical.

AUTHOR(S): Curren, R. D.

STUDY NUMBER(S): T8298.380.

REPORT ISSUED: February 10, 1989.

CONCLUSIONS: Under the conditions of the unscheduled DNA synthesis (UDS) assay in primary rat hepatocytes, five doses of Oftanol technical (0.001 to 0.03 µL/mL) did not induce a significant increase in mean net nuclear grain counts. Doses ranging from 0.03 to 3.0 µL/mL were cytotoxic and test material concentrations ≥ 1.0 µL/mL were insoluble. It was, therefore, concluded that Oftanol technical was assayed to a cytotoxic level with no evidence of a genotoxic effect.

Study Classification: The study is acceptable.

A. MATERIALS:

1. Test Material: Oftanol technical from batch No. 5030009 was described as a clear, colorless liquid with a reported purity of 91%. The test material was stored frozen (-20°C) and diluted in dimethylsulfoxide (DMSO). Solutions of the test material containing ≥ 1 µL/mL were reported to be immiscible in culture medium (Williams' Medium E).

2. Indicator Cells: Primary rat hepatocytes were harvested from the livers of adult male Fischer 344 rats obtained from Charles River Laboratories, Inc. Animals were quarantined at least 1 week prior to study initiation.

3. Cell Preparation:

   a. Hepatocyte Isolation: Each rat was anesthetized by inhalation of metofane and the livers were perfused with 0.5 mM EGTA in Hanks' buffered salt solution, pH 7.3, and Williams' Medium E (WME) containing L-glutamine (2 mM), collagenase (80 to 100 units/mL, type I), and antibiotics, buffered to pH 7.3. Livers were excised, cleaned of extraneous tissue, shaken in the collagenase perfusion solution, and either combed to release the hepatocytes or passed through a stainless-steel sieve.

   b. Hepatocyte Harvest/Culture Preparation: Recovered cells were collected, counted, and seeded at a density of 5×10^5 cells, either into preconditioned 35-mm tissue culture dishes for the cytotoxicity assay or onto coverslips in 35-mm tissue culture plates for the UDS assay. Cultures were placed in a humidified, 5% CO_2 incubator for 90 to 180 minutes, washed, and refed prior to use.

B. STUDY DESIGN:

1. Preliminary Cytotoxicity Assay: Duplicate cultures of cells, initiated from primary cultures, were exposed to 10 doses of the test material ranging from 0.0003 to 10 µL/mL, the negative control (WME), or the solvent control (DMSO) for 18 to 20 hours. Following exposure, aliquots of the treatment medium were removed, centrifuged, and measured for lactate dehydrogenase (LDH) activity. Relative cytotoxicity was assessed by subtracting the LDH activity of the media control from the LDH activity in the treated cultures and comparing the values to the amount of LDH released by exposure of high-dose cultures or solvent control cells to 1% Triton.

2. UDS Assay:

   a. Treatment/Slide Preparation: Six prepared hepatocyte cultures (three cultures seeded into tissue dishes and three cultures seeded onto coverslips) were exposed for 18 to 20 hours to eight selected doses of the test material, the negative control (WME), the solvent control (DMSO), or the positive control (3 and 10 µg/mL 7,12-dimethylbenz[a]anthracene, DMBA). Treatment medium contained 10 µCi/mL [^{3}H]thymidine. Monolayers grown directly on dishes were used to assess LDH activity as described for the cytotoxicity assay. Treated hepatocytes attached to coverslips were washed, swollen with 1% sodium citrate, fixed, (ethanol-glacial acetic acid), dried, and mounted.

   b. Preparation of Autoradiographs/Grain Development: Slides were dipped into Kodak NTB emulsion, dried for 1.5 hours, and stored at 4°C in desiccated slide boxes for 10 days. Slides were developed in Kodak D-19, fixed, stained with hematoxylin-sodium acetate-eosin, coded, and counted.

   c. Grain Counting: The nuclear grains of 150 randomly selected cells with appropriate background counts and normal morphology (50/slide) from each test, negative, and positive control group were scored for incorporation of tritiated thymidine into DNA. Net nuclear grain counts were determined by subtracting the nuclear grain count of each cell from the average cytoplasmic grain count of three nuclear-sized areas adjacent to each nucleus. Means and standard deviations were calculated for each treatment group.

3. Evaluation Criteria:

a. Assay Validity: For the assay to be considered valid, the following criteria must be satisfied: 1) the proportion of cells in repair in the solvent control must be < 15% and the net nuclear grain count of the solvent control must be < 1 and 2) the positive control compound must induce a significant increase in the net nuclear grain count (≥ 5 grains/nuclear over the negative control).

b. Positive Response: The assay was considered positive if the test material induced a dose-related increase in mean net nuclear grains and one or more of the doses had an increase in the mean net nuclear grain count that was ≥ 5 grains/nucleus over the negative control. In the absence of a dose-related effect, a compound that showed nuclear grain counts that were ≥5 grain/nucleus over two successive doses was also considered positive.

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Assay: Ten doses (0.0003 to 10 µL/mL) of the test material were examined in the cytotoxicity assay. The study author stated that the LDH values for solvent control cultures produced inconsistent results; therefore, cultures exposed to the highest test material dose (10 µL/mL) and lysed with 1% Triton were substituted as the reference data for determination of test material cytotoxicity. As shown in Table 1, cytotoxicity, as indicated by increased leakage of LDH into the culture medium, did not proceed in a conventional dose-related manner. At the highest assayed dose, <10% cytotoxicity was seen; however, as the dose was reduced, cytotoxicity increased for levels ranging from 3.0 to 0.1 µL/mL with a subsequent decline at ≤0.03 µL/mL. Percent cytotoxicity at 0.1 µL/mL was 90.9% as compared to ≈51.9% at 3 µL/mL. These results, suggesting that the physical state of high test material concentrations interfered with cellular uptake, are consistent with the study author's statement that 1, 3, and 10 µL/mL of Oftanol technical were not miscible in culture medium. At soluble levels (≤0.3 µL/mL), increased LDH activity paralleled the reported microscopic evidence of adverse morphological effects on monolayers. Based on the overall findings, 0.03 µL/mL was selected as the highest concentration for the UDS assay.

TABLE 1. Representative Results of the Preliminary Cytotoxicity Assay with Oftanol: Lactate Dehydrogenase (LDH) Activity

Treatment	Dose (µL/mL)	Average[a] LDH Activity (Units/L)	Corrected[b] LDH Activity (Units/L)	Percent[c] Cytotoxicity
Negative Control				
Culture medium	--	37.5	0.0	0.0
Solvent Control				
Dimethylsulfoxide	--	--	--	--
Dimethylsulfoxide +1% Triton	--	--	--	--
Test Material Control				
Oftanol +1% Triton	10	710.5	673.0	100.0
Test Material				
Oftanol	0.01[d]	100.5	63.0	9.4
	0.03	240.0	202.5	30.0
	0.10	649.5	612.0	90.9
	0.30	548.5	511.0	75.9
	1.00[e]	474.0	436.5	64.9
	3.00[e]	387.0	349.5	51.9
	10.00[e]	96.0	58.5	8.7

[a]Average of two samples.

[b]Corrected LDH = Average LDH of Test Groups - Negative Control LDH.

[c]Due to technical problems, LDH values for the solvent control + 1% Triton were not available for determination of percent cytotoxicity; therefore, percent cytotoxicity of test groups was determined as follows:

$$\frac{\text{Corrected LDH of Test Groups}}{\text{Corrected LDH of 10 µL/mL Oftanol + 1\% Triton}} \times 100.$$

[d]Lower doses (0.003, 0.001, and 0.0003 µL/mL) were not cytotoxic.

[e]Reported to be immiscible in tissue culture medium.

2. UDS Assay: The UDS and parallel cytotoxicity assays were conducted with eight doses (0.00003 to 0.03 µL/mL). Results presented in Table 2 show that the highest test level was 40% cytotoxic and doses ≤0.02 µL/mL induced no cytotoxic response. Accordingly, cells exposed to the five highest concentrations (0.001, 0.003, 0.01, 0.02, and 0.03 µL/mL) were evaluated for UDS. None of the doses selected for evaluation of UDS caused a significant increase in net nuclear grain counts (Table 2). By contrast, the positive control, DMBA at 3 and 10 µg/mL, showed increased levels of enzymatic and UDS activity. The study author concluded, therefore, that Oftanol technical was negative in this test system.

D. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS: We assess that the study was well conducted and that the author correctly interpreted the data. Cytotoxicity was determined by a highly sensitive method (LDH activity), which represents an improvement over gross examination of hepatocytes. The results clearly demonstrated that Oftanol technical was assayed to a cytotoxic level with no evidence of a genotoxic effect.

The ability of the test system to detect UDS was adequately shown by the findings with the positive control (DMBA at 3 and 10 µg/mL).

E. QUALITY ASSURANCE MEASURES: A quality assurance statement was signed and dated February 10, 1989.

F. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 8-10.

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TABLE 2. Representative Results of the Unscheduled DNA Synthesis Rat Hepatocyte Assay with Oftanol

Treatment	Dose /mL	Cytotoxicity: Average[a] Lactate Dehydrogenase Activity (Units/	Cytotoxicity: Corrected[b] LDH Activity (Units/L)	Cytotoxicity: Percent[c] Cytotoxicity	UDS Activity: Number Cells Scored	UDS Activity: Mean Net Nuclear Grain Count Grains ± Standard Deviations	UDS Activity: Percent Cells with >5 Net Nuclear Grains
Negative Control							
Culture medium	--	30.7	-16.0	-5	150	-1.6 ± 3.5	1
Solvent Control							
Dimethylsulfoxide	--	46.7	0.0	0	150	-1.1 ± 2.7	1
Dimethylsulfoxide + 1% Triton	--	343.7	297.0	100	150	--	--
Positive Control[d]							
7,12-Dimethyl-benz(a)anthracene	3 µg	87.7	41.0	14	150	14.2 ± 5.1*	100
Test Material							
Oftanol	0.02 µL[e]	60.0	13.3	4	150	-1.4 ± 2.6	1
	0.03 µL	166.0	119.3	40	150	-0.9 ± 2.5	1

[a]Average of three samples.

[b]Corrected LDH = Average LDH of Test Group - Negative Control LDH.

[c]Percent Cytotoxicity = $\frac{\text{Corrected LDH of Test Group}}{\text{Corrected LDH of Solvent Control Cultures Exposed to 1\% Triton.}}$.

[d]Two levels were assayed; the lowest level was selected as representative.

[e]Results for lower doses (0.01, 0.003, and 0.001 µL/mL) were comparable to the solvent control values.

*Conforms to the reporting laboratory's criteria for a positive response.

APPENDIX A
Materials and Methods
(CBI pp. 8-10)

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EPA No.: 68D80056
DYNAMAC No.: 175-A
TASK No.: 1-75A
May 8, 1989

DATA EVALUATION RECORD

OFTANOL

Mutagenicity--In vitro Cytogenicity Assay in
Chinese Hamster Ovary (CHO) Cells

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: [signature]

Date: 5/8/89

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EPA No.: 68D80056
DYNAMAC No.: 175-A
TASK No.: 1-75A
May 8, 1989

DATA EVALUATION RECORD

OFTANOL

Mutagenicity--In vitro Cytogenicity Assay in Chinese Hamster Ovary (CHO) Cells

REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy L. McCarroll
Date: 5-8-89

I. Cecil Felkner, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: [signature] for I. Cecil Felkner
Date: 5-8-89

APPROVED BY:

Roman J. Pienta, Ph.D.
Department Manager
Dynamac Corporation

Signature: Roman J. Pienta
Date: 5-8-89

Mike Ioannou, Ph.D.
EPA Reviewer and
Acting EPA Section Head
Section II
Toxicology Branch I (H-7509C)

Signature: M. Ioannou
Date: 5-10-89

DATA EVALUATION RECORD

CHEMICAL: Oftanol.

STUDY TYPE: In vitro cytogenetic assay in Chinese hamster ovary (CHO) cells.

MRID NUMBER: 410088-01.

TEST MATERIAL: Oftanol technical.

SYNONYM/CAS no: 25311-71-1.

SPONSOR: Mobay Corp., Stilwell, KS.

TESTING FACILITY: Microbiological Associates, Inc., Rockville, MD.

TITLE OF REPORT: Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells.

AUTHOR(S): Putman, D. L., and Morris, M. J.

STUDY NUMBER(S): T8298.337

REPORT ISSUED: February 6, 1989.

CONCLUSIONS - Executive Summary: Oftanol technical, assayed with and without metabolic activation over a dose range of 0.02 to 0.16 µL/mL, induced cytotoxic effects in Chinese hamster ovary (CHO) cells, but was not clastogenic.

Study Classification: The study is acceptable.

A. MATERIALS:

1. Test Compound:

a. Description: Oftanol technical from batch No. 5030009, formula No. 011520, was described as a clear, colorless, viscous liquid with a reported purity of 91%. The test material was stored frozen (-20°C) and protected from light.

b. Solubility/Osmolality Determinations: The test material was soluble in dimethylsulfoxide (DMSO). The highest soluble dose was not specified, but the authors indicated that 0.16 µL/mL of the test material were partially soluble in culture medium (McCoy's 5A medium). The osmolality of the highest assayed dose (0.16 µL/mL) was determined and found to be 449 mOsm/kg.

2. Cell Line: The Chinese hamster ovary cells (CHO-K_1) used in this assay were obtained from the American Type Culture Collection, Rockville, MD. Prior to use, the CHO cells were grown for 16 to 24 hours in McCoy's 5A medium supplemented with 10% fetal calf serum, L-glutamine, and antibiotics.

3. S9 Fraction: The S9 fraction was derived from the livers of adult male Sprague-Dawley rats induced with Aroclor 1254. Following preparation, the ability of the fraction to metabolize 2-aminoanthracene and 7,12-dimethylbenz(a)-anthracene to mutagenic forms was assessed in Salmonella typhimurium TA100. The S9 reaction mixture contained 15 µL/mL rat liver S9.

B. STUDY DESIGN:

1. Preliminary Cytotoxicity Assay: Prepared cultures, seeded at 5×10^5 cells/flask, were exposed with or without S9 activation to nine concentrations of the test material (0.16×10^{-4} to 0.16 µL/mL) or the solvent control (DMSO).

In the nonactivated system, cells were exposed for 6 hours to the test material; 50 µL of 1 mM BrdU were added to the cultures 2 hours after initiation and incubation was continued. In the S9-activated system, cultures were treated for 2 hours. After exposure, cells were washed, refed with complete medium containing BrdU (0.01 mM), and reincubated for a total of 24 hours.

Two hours prior to the end of incubation, colcemid, at a final concentration of 0.1 µg/mL, was added to each culture. Metaphases were harvested, fixed, and stained with the modified fluorescent-plus-Giemsa technique of Perry and Wolff.[1] One hundred cells from each dose group were examined for the percentage of first division (M_1), second division (M_2), and third division (M_3) metaphases. Mitotic indices were calculated as the percentage of mitotic cells in 500 cells scored per dose.

Based on these results, dose selection and the maximum harvest time for the cytogenetic assay were established.

2. Cytogenetic Assay:

   a. Treatment: Prepared cultures (in duplicate), seeded at 5 x 10^5 cells, were exposed to the selected doses of the test material, the solvent control (DMSO), or the positive controls, 0.5 µg/mL triethylenemelamine (TEM) -S9 and 50 µg/mL cyclophosphamide (CP) +S9.

      In the nonactivated assay, cells were dosed for 18 hours. Cultures were washed, refed medium containing 0.1 µg/mL colcemid, and reincubated for 2 hours. Under S9-activated conditions, cells were exposed for 2 hours, washed, refed culture medium, and incubated for an additional 16 hours. Colcemid (0.1 µg/mL) was added 2 hours before the cultures were harvested.

      Metaphase cells were collected and fixed. Slides were stained with 5% Giemsa and coded.

[1]Perry, P., and Wolff, S. New Giemsa method for the differential staining of sister chromatids. Nature (1974) 251: 156-158.

b. Metaphase Analysis: One hundred metaphase cells per group (50/culture) were scored for chromosome aberrations. Chromatid and chromosome gaps were counted, but not included, in the final analysis. Mitotic indices were calculated.

3. Statistical Methods: The data were evaluated for statistical significance by Fisher's exact and/or Cochran-Armitage tests at p values of 0.05 and 0.01.

4. Evaluation Criteria:

a. Assay Validity: The assay was considered valid if the percent of cells with aberrations in the untreated control did not exceed 6% and the number of cells with aberrations in the positive control was significantly higher ($p \leq 0.05$) than in the solvent control.

b. Positive Response: The test material was considered positive if it caused a dose-related increase in the percentage of cells with aberrations and one or more doses were significantly increased ($p<0.05$) relative to the solvent control.

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Assay: The nine nonactivated and S9-activated test material doses evaluated in the preliminary cytotoxicity assay ranged from 0.16×10^{-4} to 0.16 µL/mL. As shown in Table 1, the two highest nonactivated and S9-activated doses (0.048 and 0.16 µL/mL) caused severe mitotic delay; mitotic indices were markedly reduced in cultures exposed to 0.16 µL/mL +/-S9. No appreciable effect on cell-cycle kinetics was evident at nonactivated and S9-activated dose ≤0.016 µL/mL of the test material. Based on these results, the four dose levels selected for the cytogenetic assay were 0.02, 0.04, 0.08, and 0.16 µL/mL. Due to the effects on cell-cycle kinetics, cultures were harvested 20 hours postexposure to the selected test material doses.

2. Cytogenetic Assays: The study authors reported that at the time of harvest, the three highest doses without S9 activation (0.04, 0.08, and 0.16 µL/mL) and the two highest S9-activated doses had a slight cytotoxic effect on the treated monolayers. Mitotic indices were reduced at all nonactivated doses and at the highest S9-activated treatment level; however, no increase in the frequency of aberrations per cell or percentage of cells with

TABLE 1. Representative Results from the Preliminary Cytotoxicity Assay with Oftanol

Substance	Dose (μL/mL)	S9 Activation	% Cells[a] M_1	% Cells[a] M_2	Mitotic[b] Index
Solvent Control					
Dimethylsulfoxide	--	-	0	100	5.8
	--	+	2	98	5.8
Test Material					
Oftanol	0.016[c]	-	0	100	4.8
	0.048	-	96	4	2.6
	0.16	-	100	0	1.4
	0.016[c]	+	4	96	3.8
	0.048	+	55	45	5.8
	0.16	+	100	0	1.0

[a]Percent cells in first (M_1) or second (M_2) division; no third division cells were seen.

[b]Number of metaphase cells per 500 cells scored.

[c]Lower doses (0.48×10^{-2} to 0.16×10^{-4} μL/mL/+ or -S9) had no appreciable cytotoxic effects.

aberrations were scored in cultures exposed to 0.02, 0.04, 0.08, or 0.16 µg/mL of the test material either in the preserce or absence of S9 activation. By contrast, TEM at 0.5 µg/mL/-S9 and CP at 50 µg/mL/+S9 induced significant ($p < 0.01$) increases in the percentage of cells with aberrations.

Representative results are shown in Table 2. Based on these findings, the study authors concluded that Oftanol technical was not clastogenic in this test system.

D. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS: We assess that the study was properly conducted and that the authors interpreted the data correctly. In both the presence and absence of S9 activation, Oftanol technical was assayed up to cytotoxic levels with no indication of a clastogenic effect. By contrast, both the nonactivated and S9-activated positive controls induced significant ($p < 0.01$) increases in chromosome aberrations, indicating that the sensitivity of the assay to detect a clastogenic response was adequate.

E. QUALITY ASSURANCE MEASURES: A quality assurance statement was signed and dated February 9, 1989.

F. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 8-12.

TABLE 2. Representative Results of the CHO Cell *in vitro* Cytogenetic Assay with Oftanol Technical Following a 20-Hour Harvest

Substance	Dose/mL	S9 Activation	No. of Cells Scored	% Cells with Aberrations	Aberrations per Cell ± Standard Deviations	Mitotic Index
Negative Control						
Untreated cells	--	-	100	3	0.03 ± 0.17	4.5
		+	100	1	0.01 ± 0.10	8.1
Solvent Control						
Dimethylsulfoxide	--	-	100	4	0.04 ± 0.20	4.2
		+	100	3	0.03 ± 0.17	6.7
Positive Control						
Triethylenemelamine	0.5 µg	-	100	30*	0.59 ± 1.55	2.6
	50.0 µg	+	100	15*	0.18 ± 0.46	4.0
Test Material						
Oftanol	0.16 µL[a]	-	100	0	0.00 ± 0.00	1.5
		+	100	2	0.02 ± 0.14	4.6

[a]Slight toxic effects were reported on monolayer cultures treated with this level and at lower test material doses (0.04 and 0.08 µL/mL/-S9 and 0.08 µL/mL/+S9); however, the frequency of chromosome aberrations for all test doses, with or without S9-activation, was comparable to the corresponding solvent control results.

*Significantly higher than the solvent control ($p < 0.01$) by Fisher's exact test.

APPENDIX A

Materials and Methods

Tox # 007192

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